REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application, in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

As noted in the Office Action of November 23, 2004, claims 18-45 are pending. Claim 33 is amended herein to address issues of grammar, and thus no prohibited new matter is submitted herein.

Applicants note with appreciation that the previous rejections under 35 U.S.C. §§ 102 and 103, as well as objections to the specification, have been withdrawn. Applicants further note with appreciation the acknowledgement of Applicants' claim of priority to U.S. Patent Application Nos. 08/667,493 and 08/311,553 on page 2 of the Office Action, and the notation by the Office that the drawings are considered acceptable.

Applicants request an interview with the Examiner following the filing of the present Reply, in order to discuss remaining issues. Applicants will contact the Examiner accordingly.

Claim Rejections Under 35 U.S.C. § 103

A. Rejection of claims 18-36 and 42-45 over Ben-Ezra in view of Shibata and McKenzie

Claims 18-36 and 42-45 stand rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Ben-Ezra et al. (*J. Histochemistry and Cytochemistry*, 39: 351-354 (1991)) ("Ben-Ezra") in view of Shibata et al. (*Am. J. Pathology*, 121: 539-543 (1992)) ("Shibata") and McKenzie et al. (U.S. Patent No. 5,491,062)

("McKenzie"). Ben-Ezra is cited as purportedly disclosing the effect of fixation on the amplification by PCR of nucleic acids from paraffin-embedded material, and that amplification by PCR of nucleic acids from paraffin-embedded material is increasingly being used to detect viral genomes and oncogene mutations. *See* Office Action dated November 23, 2004, at 3. However, the Office acknowledges that Ben-Ezra does not disclose the analysis of a biological specimen under a microscope for the selection of a target based on histopathological characteristics and placing the targeted cells on a slide. The Office further acknowledges that Ben-Ezra does not disclose a step of centrifugation following a step of boiling extraction to obtain a DNA containing supernatant and cycling the PCR reaction for five minutes. *Id.* at 4.

The Office states that allegedly Shibata discloses a method of specific genetic analysis of microscopic tissue after selective ultraviolet radiation fraction and the polymerase chain reaction. Shibata is also cited for allegedly teaching a method of placing a biological specimen having DNA of a patient under a microscope. *Id.* at 4-5. McKenzie is cited for purportedly disclosing centrifuging an aqueous solution from the extraction and creating a pellet and a DNA-containing supernatant and using the supernatant for the PCR reaction. The Office states that it would have been *prima facie* obvious to have modified the DNA extraction and analysis method of Ben-Ezra with the teachings of Shibata (targeting of particular specimens) and McKenzie (pelleting the cellular debris) to obtain the invention as a whole. *Id.* at 6-7. Applicants respectfully traverse.

For a *prima facie* case of obviousness, the following three requirements must be met. First, the prior art relied upon, coupled with the knowledge generally

available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the skilled artisan to modify a reference or to combine the reference with another reference. Second, the proposed modification must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. Third, the prior art reference must teach or suggest all the limitations of the claims. The teachings or suggestions, as well as the expectation of success, must come from the prior art and not from applicant's disclosure. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988); *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1209, 18 U.S.P.Q.2d 1016, 1023 (Fed. Cir. 1991); and *In re Vaeck*, 947 F.2d 488, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991).

Applicants respectfully submit that the cited references, alone or in combination, do not meet the requirements for a *prima facie* case of obviousness. There is no motivation to modify the teachings of Ben-Ezra, alone or in view of Shibata and McKenzie, for the claimed purpose. Nor is there an expectation that such a modification would lead to the successful methods of topographical genotyping of the present invention. The Office is picking and choosing elements from three difference references in an attempt to arrive at the claimed method. In addition, these references are not properly combined, as they disclose inefficient methods and would not work in combination. The Office never meets any standard other than at best that of "obvious to try", which is not the standard by which a *prima facie* case of obviousness is determined.

To set forth a case of *prima facie* obviousness, a reference must be viewed as a whole for what it teaches; "[i]t is impermissible within the framework of section 103

to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *In re Wesslau*, 353 F.2d 238, 241, 147 U.S.P.Q. 391, 393 (C.C.P.A. 1965); *see also Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986). In the present case, the Office picks and chooses from the references in the attempt to gather all of the elements of the present invention together, ignoring what each reference teaches as a whole. The failings of the references, alone and in combination, are addressed in detail below.

Ben-Ezra - The Primary Reference

Ben-Ezra is dated 1991, when polymerase chain reaction (PCR) technology was still being developed. The disclosure of Ben-Ezra is an early study of the efficacy of PCR when performed on DNA and RNA from a fixed sample. However, this reference does not disclose the element of the present invention cited by the Office, and actually teaches away from the present invention.

First, Ben-Ezra discloses a much larger piece of tissue than that used in the present invention. In Ben-Ezra, the whole tissue section (6 microns thickness) is taken into the tube. This is in sharp contrast to the present invention, where a microdissected portion of the tissue sample (*i.e.*, very small) is required in order to target a very precise area of tissue (for example, in detecting the early development of cancer). With regard to diameter, Ben-Ezra describes its tissue sample as breast tissue, *i.e.*, fibroadenoma, about 2 centimeters in diameter. In comparison, the diameter of the tissue samples of the present invention are far smaller, about 1

millimeter in diameter, Applicants further point out that the samples are further microdissected from this small size. Thus, Ben-Ezra uses about 4000 times more tissue as is used in the present invention.

Second, Ben-Ezra discloses placing the large section of tissue into 50 microliters of buffer solution for use in a PCR reaction. Ben-Ezra then discloses using 45 microliters of this mixture in the PCR reaction. Thus, Ben-Ezra is basically using the entire sample for just one PCR reaction. In contrast, the present invention only requires 1 microliter of the sample for the PCR reaction. This is a distinct advantage over Ben-Ezra, as 15-20 separate PCR reactions can be performed on tissue from the same specimen and are required to perform the mutational profiling. Because Ben-Ezra uses his entire sample, no mutational analysis can be performed.

Finally, as using the relatively new technique of PCR, Ben-Ezra discloses PCR reactions on both DNA and RNA. However, Ben-Ezra teaches that the results obtained with RNA were seen to better than those with DNA. Thus, Ben-Ezra provides motivation to amplify RNA, not DNA. *See* page 252, second column. Also, while Ben-Ezra discloses that deparaffinized material may be subjected to proteinase K digestion with or without phenol/chloroform extraction, the author notes that this technique was not actually used. *See* page 353. Rather, crude, boiled extracts were used. Ben-Ezra goes on to explain that the use of the boiled extract may have contributed to poor PCR results, as a result of protein contamination. Thus, Ben-Ezra does not provide motivation for the skilled artisan to use DNA in the reaction.

Shibata Does Not Cure The Defects Of Ben-Ezra

Shibata discloses the use of selective ultraviolet (UV) radiation fractionation in combination with PCR to analyze cell subsets present on a microscope section. Specifically, cells of interest are selected and then separated from background cells. UV radiation is used to destroy all cells except the cells of interest, which are protected by a physical barrier. *See* page 539, column 2.

Shibata uses a very different technique than that claimed here. Additionally, Shibata fails to remedy the deficiencies of Ben-Ezra. The technique disclosed by Shibata is known as SURF (Selective genetic analysis of microscopic tissue heterogenicity). In the SURF technique, 4 micron thick tissue sections are placed on acetate sheets, instead of glass slides. Next, a felt tipped pen is used to place an ink dot "precisely" on the microscopic area of interest, thereby forming an umbrella-like dome over the DNA. However, dried tissue is highly absorbent, and the natural osmotic action of the tissue sample absorbs the ink drop in an uncontrolled fashion, and so it is almost impossible to place the ink drop and confine it to a specific area.

Next, the tissue is exposed to UV light, in order to burn the "uncovered" regions such that the unprotected DNA is destroyed, while the dotted area is protected. Then, the acetate is cut up into pieces and the DNA is extracted.

Shibata is cited for purportedly disclosing placing a tissue section in a tube and then performing direct multiple PCR reactions. However, the skilled artisan would not have known at the time which of the many steps of Shibata are to be combined with the many steps of Ben-Ezra. Shibata is further distinguishable; it describes the targeting of human papilloma virus (HPV), which is present in very

large amounts in infected cells. There is so much virus present in the infected cells that Shibata does not have to be efficient. Also, Shibata discloses running the HPV from the sample through agarose, and further discloses the location of the HPV by using ethidium bromide, as there was so much virus present. However, they were unable to view the single copy p53 in agarose. Thus, the method of Shibata is not effective for determining minute quantities of DNA due to mutational changes in genomic DNA.

As with Ben-Ezra, the reactions of Shibata were inefficient, especially in comparison to the efficiency of the present invention. Taken alone, these two cited references lead the skilled artisan to use techniques that result in extremely ineffective PCR. When combined, the references provide no guidance as to which of each method's disparate steps were to be combined or that the steps should even have been combined. The references must be viewed as a whole for their teachings, and steps cannot be chosen and recombined with steps from another's teaching, in isolation of the teachings of the references as a whole.

McKenzie Does Not Cure The Defects Of Ben-Ezra Or Shibata

McKenzie is directed to the detection of mycoplasma infections in cell cultures and animals. McKenzie discloses polynucleotide primer pairs, which can hybridize to mycoplasma tRNA genes to generate amplification products used in PCR. McKenzie is cited merely to show centrifugation of an aqueous solution to create a pellet and DNA supernatant, wherein the supernatant then may be used in a PCR reaction.

McKenzie does not utilize fixative treated tissues or cells, but instead uses fresh cell cultures. Nothing in McKenzie motivates the skilled artisan to use fixative treated specimens. Thus, the Office again seeks to select a step and isolate that step from the teachings of the rest of the reference in which the step is found and combine it with similarly isolated steps from other references. The methods of McKenzie are again highly inefficient, even with fresh cell cultures. If the McKenzie teachings were applied to fixed specimens, it would result in even greater inefficiency. The skilled artisan would not have been motivated to extrapolate McKenzie's teachings to fixed tissue, with the methods of the other two references.

McKenzie discloses using one million cultured, fresh cells. This number of cells is at least three times more than those used with the microdissection-based analysis of fixed tissue, as with the presently claimed invention. Thus, McKenzie's technique requires a far greater number of target DNA templates than would present in the fixed tissue of the claimed method. Additionally, McKenzie requires purified DNA that can be accurately measured. On page 9, line 23, McKenzie states that the concentration of DNA should be adjusted to a desired level. However, this adjustment is not possible in fixative treated tissues. Therefore, the methods of McKenzie would not be effective when used on fixed tissue samples. In summary, McKenzie cannot be combined with the other references, and the deficiencies of the other references are not addressed by McKenzie.

The Cited References Are Not Properly Combined

In an effort to locate all of these elements, the Office has combined the three references. However, the mere fact that references can be combined or modified

does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990). *See* also *In re Fritch*, 972 F.2d 1260, 23 U.S.P.Q.2d 1780. Although the motivation need not be explicit, the motivation must be present to combine the prior art references in a manner to solve the problem. *See Ruiz v. A.B. Chance Co.*, 69 U.S.P.Q.2d 1686, 1690-91 (Fed. Cir. 2004). The currently claimed method which solves the problem of being unable to detect mutations in minute sections of fixed tissue is not taught by the references. The references each address different problems, which include (1) methods of identifying the presence of a mycoplasma in fresh cells not fixed cells (McKenzie); (2) utilizing an entirely different method of isolating DNA involving UV treatment and acetate platforms to work with fixed tissue, wherein the tissue again is being analyzed for the presence of human papilloma virus (HPV) which is present in the sample in large quantities (Shibata); and (3) using a fixed sample that is 4000X the size of the samples of the claimed method in a single reaction, which cannot be used for mutational profiling of multiple mutations (Ben-Ezra).

The Office has failed to explain what specific understanding or technological principle within the knowledge of one of ordinary skill in the art would have suggested the particular combination deduced by the Office. Absent such an explanation of the specific understanding or principle within the knowledge of the skilled artisan at the time that would have motivated that artisan with no knowledge of the Applicants' invention to make the claimed method, Applicants respectfully submit that the selected references were chosen with the assistance of hindsight. This conclusion is reached because the references address different problems in the

field and do not related to problem being solved by the claimed invention. Hindsight is forbidden in the selection of references that comprise the case of obviousness. *In re Rouffet*, 47 U.S.P.Q.2d 1453, 1458 (Fed. Cir. 1998). Three possible sources for motivation to combine prior art references in manner that would render the claimed invention obvious are nature of the problem to be solved, the teachings of the prior art, and knowledge of persons of ordinary skill in the art. A high level of skill in the art cannot be relied upon to provide the motivation to combine absent explanation for what specific understanding or technical principle would have suggested the combination. *See id.*, at 1453. As noted in *Rouffet*, the Federal Circuit stated that a high level of skill in the art is not enough to compensate for a lack of motivation to combine. In the present case, the Office does not even cite to a high level of skill in the art. Instead, the Office merely uses a level of ordinary skill in the art to provide the missing motivation to combine. When viewed alone and as a whole, each reference fails to teach the currently claimed method. When viewed in combination, each reference as a whole fails to provide guidance or motivation to combine the steps selected by the Office in its attempt to adduce a rejection of *prima facie* obviousness. As the Court reasoned in *Rouffet*, absent such a teaching and given the disparity of the references, there is no motivation to combine the references in the asserted manner. Accordingly, there cannot be an expectation of success that such a combination would work.

B. Rejection of claims 37-41 over Ben-Ezra in view of Shibata, McKenzie, and Perlin

Claims 37-41 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Ben-Ezra in view of Shibata and McKenzie and further in view of Perlin (U.S. Patent No. 5,580,728, December, 1996) ("Perlin").

The Office acknowledges that Ben-Ezra, Shibata, or McKenzie fail to disclose using a database for analyzing the results of genotyping. However, Perlin purportedly teaches a method and system for genotyping which comprises a computer and a database. Perlin also purportedly discloses this use of computers with respect to phenotypic risk of disease for the individual, genetic linkage maps, genome maps, cloning a disease gene, treating disease, monitoring cancerous materials, fingerprinting, performing population genotyping studies, and assessing genetic risk. Thus, the Office states that it would have been obvious to have modified the genotyping method of Ben-Ezra, Shibata, and McKenzie to include a computer/database as taught by Perlin.

However, Perlin is cited merely to include the element of the use of computers and databases. It does not remedy the deficiencies of the other three references, as discussed *supra*. Nor does Perlin provide motivation to combine any of the references. Thus, as it fails to address these issues, it does not render claims 37-41 obvious over the cited references.

C. Unexpected Results

Further, Applicants respectfully submit that the claims are patentable over the cited references because unexpected results are present with respect to the claimed methods.

It is a well established legal precedent that the presence of an unexpected, advantageous or superior result is evidence of nonobviousness. *See, e.g.,* M.P.E.P. § 716.02(a); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (C.C.P.A. 1963). Along these lines, it is also well established that "a greater than expected result" is evidence of nonobviousness. *See* M.P.E.P. § 716.02(a); *In re Corkill*, 711 F.2d 1496, 226 U.S.P.Q. 1005 (Fed. Cir. 1985).

As noted in the present specification, the rate limiting step when handling fixative treated tissue specimens for genetic analysis is effective and specific DNA amplification. At the time, DNA that has been exposed to chemical fixatives was often unsuccessfully or only poorly amplified. Previously, the investigator had to sacrifice large amounts of fixative treated tissue or abandon the use of fixative treated tissues altogether.

However, surprisingly, it is a misconception that fixative treated tissue provides an inadequate amount of starting DNA for nucleic acid amplification. In reality, the tissue obtained from a 1-4 micron thick histologic section of a small biopsy specimen, when handled properly, provides sufficient material for consistent and effective DNA amplification. In fact, when one attempts to add more fixative treated tissue to an amplification reaction, the results of the amplification are often poor. Applicants have discovered that it is actually important to use a small rather than a large amount of tissue to initially trigger the amplification reaction. Once triggered in the first few cycles to copy a sufficient quantity of DNA from the tissue template, the remainder of the amplification reaction goes forth in the buffer solution without significant participation of the original tissue DNA. This discovery is contrary to what is disclosed in the art. For example, the cited reference of Ben-Ezra

requires a large sample of tissue (*i.e.*, at least 4000 times greater sample than the sample used in the claimed method) in order to get a sufficient amount of DNA for a PCR reaction.

In light of the above remarks, Applicants request that the rejections under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the application may be expedited.

If there are any questions concerning this paper or the application in general, Applicants invite the Examiner to telephone the undersigned at the Examiner's earliest convenience.

In the event any further fees are due to maintain pendency of this application, the Examiner is authorized to charge such fees to Deposit Account No. 02-4800.

Respectfully submitted,

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